

ELECTROCHEMICAL ENZYME ASSAY

CROSS-REFERENCES TO RELATED APPLICATIONS

This is a continuation-in-part of application Ser. No. 08/113,548, filed Aug. 27, 1993, which is now U.S. Pat. No. 5,427,912, issued Jun. 27, 1995.

FIELD OF THE INVENTION

This invention relates generally to the field of electrochemical enzyme assays.

BACKGROUND OF THE INVENTION

Radioimmunoassay was developed in 1960 by Yalow and Berson as a method for detecting or quantitating antigens or antibodies using radiolabeled reactants. Since the initial studies in 1960, radioimmunoassay (RIA) has developed into a versatile analytical technique, particularly useful in clinical laboratories to quantitate a wide variety of compounds. With RIA, the unknown concentration of an unlabeled antigen is determined by comparing its inhibitory effect on the binding of a radioactively-labeled antigen to an antibody. RIAs do have a number of significant limitations, however, including a limited shelf-life, high cost, and potential environmental harm.

The disadvantages associated with RIAs led to the development of the enzyme immunoassay (EIA), in which the activity of an enzyme is measured to quantitate an analyte. EIAs are subdivided into heterogeneous assays and homogeneous assays. Heterogeneous EIAs require a physical separation of the antibody-bound, labeled analyte from the unbound labeled analyte. With homogeneous EIAs, a separation step is not required. Homogeneous EIAs have been successful commercially because of their speed, simplicity, and automation. The enzymatic activity associated with EIAs is often monitored spectrophotometrically, using a substrate which produces a unique chromophore as a result of an enzymatic reaction.

In addition to using spectrophotometric detection techniques, EIAs have been developed which use electrochemistry to monitor activity of the enzyme label. With electrochemical detection, the active enzyme causes the formation of an active electron mediator or a redox couple from an inactive substrate. The activated mediator or redox couple then shuttles electrons from the enzyme to the electrode or from the electrode to the enzyme. The resulting current can be measured and correlated to analyte level.

Direct electrochemical enzymatic assays (non-immunological) are also known in which the presence or absence of the analyte to be measured causes an electroactive compound to be cleaved from a non-electroactive substrate. The electroactive compound may then be oxidized or reduced and the resulting current measured.

Enzyme complementation immunoassays have also been developed, such as CEDIA® (Cloned Enzyme Donor ImmunoAssay—a registered trademark of the Microgenics Corporation) technology, an example of which is described in U.S. Pat. No. 4,708,929 (issued Nov. 24, 1987), which is hereby incorporated by reference. CEDIA® technology involves the use of enzyme acceptor and enzyme donor polypeptides prepared by recombinant DNA techniques or synthetic peptide synthesis techniques which are capable of spontaneously associating in solution to form an active enzyme complex. This association can be modulated, for

example, by conjugating the enzyme donor polypeptide to a member of a specific binding pair, and providing the complementary member of the specific binding pair elsewhere in the assay. The enzyme donor polypeptide may also be chemically modified to include a specific recognition site that is not a member of a specific binding pair (e.g., a protease site or an esterase site). Accordingly, in its broadest sense, CEDIA® technology allows the formation of an active enzyme complex by the spontaneous association of enzyme acceptor and enzyme donor polypeptides to be dependent on the presence or concentration of an analyte of interest. The amount of enzymatic activity is then monitored spectrophotometrically.

One embodiment of CEDIA® technology is shown in FIG. 1. Analyte analog **1** is covalently attached to enzyme donor polypeptide **2** to form enzyme donor polypeptide conjugate **3**. Analyte-specific antibody **4** can be used to inhibit reassembly of enzyme donor polypeptide conjugate **3** with enzyme acceptor polypeptide **6**. When a sample containing analyte **8** is introduced, analyte **8** and enzyme donor polypeptide conjugate **3** compete for binding to antibody **4**. As the amount of analyte **8** increases, less enzyme donor polypeptide conjugate **3** binds to antibody **4** and more active enzyme **10** is formed. Active enzyme **10** hydrolyzes enzyme substrate **11** (e.g., chlorophenol-red- β -D-galactopyranose (CPRG)), which then undergoes a color change and is monitored spectrophotometrically.

SUMMARY OF THE INVENTION

The present invention is based on the novel combination of CEDIA® technology (i.e., the modulation of enzyme activity in response to the presence or concentration of an analyte) with electrochemical detection of the resulting enzyme activity in order to determine the presence or concentration of the analyte. The advantages that result from this combination include the speed and simplicity of a homogeneous EIA and the simplicity, enhanced analyte sensitivity, small sample volume requirement, and adaptability to sensor formats associated with electrochemical measurement of enzyme activity.

The assay components include an enzyme acceptor polypeptide (EA), an enzyme donor polypeptide (ED), a substrate for enzymatic reaction, and a label which is bound to the substrate and is preferably nonelectroactive until cleaved from the substrate. ED is capable of combining with EA to form an active enzyme complex, the formation of the active enzyme complex being responsive to the presence or concentration of an analyte in a sample.

The sample containing the analyte is mixed with a first reagent (EA reagent) which includes EA. This mixture is then mixed with a second reagent (ED reagent) which includes ED and the labeled substrate. The enzyme activity resulting from the combination of EA and ED is responsive to the presence or concentration of the analyte. The active enzyme then cleaves the label from the substrate, which may be detected electrochemically. The current measured from the oxidation of the label may then be correlated to the concentration of the analyte in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of the assay components of one embodiment of CEDIA® technology.

FIG. 2 is a block diagram of one embodiment of the present invention.